

The University of Chicago

PARTIAL SYNTHESIS OF
COMPOUNDS RELATED TO ADRENAL
CORTICAL HORMONES

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

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SOME REACTIONS OF AN EPOXIDE OF Δ^{11} -LITHOCHOLENIC ACID*

By WILLIAM P. LONG

The preparation of Δ^{11} -lithocholenic acid by Kendall and coworkers (1) and by Press and Reichstein (2) yields an important substance for the partial synthesis of steroid compounds related to adrenal cortical hormones. We have investigated certain reactions of the epoxide of this compound, since it seemed desirable to explore the possibility of introducing an oxygen function at C-11 of the steroid nucleus by way of this derivative.

Both Kendall (1) and Press and Reichstein (2) have described an 11,12 epoxide prepared by the action of perbenzoic acid on Δ^{11} -lithocholenic acid or its derivatives. Kendall did not assign configuration to the epoxide. Press and Reichstein (2) designated the product 11(β),12(β)-epoxy because of the fact that desoxycholic acid (in which the hydroxyl group at C-12 has β configuration according to Reichstein and Sorkin (3)) can be isolated when the epoxide is reduced by hydrogen in the presence of Raney nickel under 150 atmospheres at 100°. Since the configuration of the hydroxyl group at C-12 in desoxycholic acid is unsettled (4), it seemed undesirable to assign a definite configuration to the epoxide on the basis of these results.

Kendall¹ in earlier work had noted that when 3(α)-hydroxy-11,12-epoxy-cholanic acid (1) was dissolved in acetic acid there was an immediate positive shift in rotation upon the addition of hydrobromic acid. Since the action of mineral acid on epoxides can lead to many compounds, depending upon the nature of the acid, the solvent, temperature, and other variables, it seemed desirable to investigate the reaction under precisely defined conditions. For the purpose of following qualitatively the course of reaction we have made use of the considerable changes in optical rotation which accompany the transformations of the epoxide. Although not all of the possible products have been identified, our experiments indicate that the principal chemical changes involved when an acetone or acetic acid solution of the

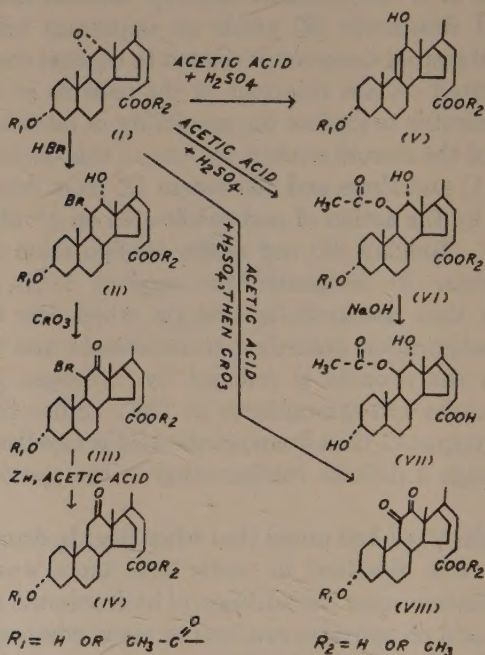
* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

¹ Kendall, E. C., personal communication.

11,12 epoxide is treated with hydrobromic or sulfuric acid are the introduction of the anionic constituent at C-11 and the formation of a hydroxyl group at C-12. The stability of the substituent at C-11 is, however, markedly influenced by the experimental conditions.

EXPERIMENTAL²

Methyl 3(α)-Acetoxy-11,12-epoxycholanate (I, $R_1 = \text{CH}_3-\overset{\text{O}}{\parallel}\text{C}-$; $R_2 = \text{CH}_3$)—This was prepared by treating a chloroform solution of methyl 3(α)-acetoxy- Δ^{11} -cholanate (m.p. 118–120°) with excess perbenzoic acid in



chloroform at room temperature. The reaction was rapid and quantitative. The chloroform was washed with sodium carbonate solution and with water, and, after drying with Na_2SO_4 , the solvent was removed in a partial vacuum. Crystallized from acetone the epoxide formed prisms melting at 145°; $[\alpha]_D^{22} = +57^\circ$ (acetone or acetic acid); $[\alpha]_D^{23} = +26^\circ$ (toluene).

$\text{C}_{27}\text{H}_{42}\text{O}_6$. Calculated, C 72.61, H 9.48; found (T. S. M.), C 72.49, H 9.17

² The microanalyses were performed by Dr. T. S. Ma (T. S. M.), Department of Chemistry of the University of Chicago, and by Dr. Amel Menotti (A. M.), American Medical Association, Chicago, Illinois. We wish to express our appreciation for this service. All melting points are corrected.

Methyl 3(α)-Hydroxy-11,12-epoxycholanate (I, $R_1 = H$; $R_2 = CH_3$)—This was prepared in a similar fashion to that of the previous compound from methyl 3(α)-hydroxy- Δ^{11} -cholenate (m.p. 101–103°). Upon recrystallization from petroleum ether and from dilute methanol it formed clusters of small plates which melted at 101–103°; $[\alpha]_D^{21} = +44^\circ$ (acetone or acetic acid); $+12^\circ$ (toluene).

3(α)-Hydroxy-11,12-epoxycholanic Acid (I, R_1 and $R_2 = H$)—This was prepared by saponification at room temperature with aqueous alcoholic KOH of either of the two preceding products. After recrystallization from ethyl acetate-petroleum ether, the compound formed needles, m.p. 164–165°; $[\alpha]_D^{25} = +44^\circ$ (acetic acid); $+40.2^\circ$ (acetone or ethanol); $+20.5^\circ$ (toluene).

Methyl 3(α)-Acetoxy-11(β)-bromo-12(α)-hydroxycholanate (II, $R_1 =$
 $\begin{array}{c} O \\ // \\ CH_3-C-; R_2 = CH_3 \end{array}$)—1.109 gm. of methyl 3(α)-acetoxy-11,12-epoxycholanate were dissolved in 20 ml. of redistilled acetone. 5.0 ml. of 0.72 N hydrobromic acid in acetone were added and the mixture allowed to stand at room temperature for 15 minutes. In this experiment the rotation was not measured but in a number of other instances the specific rotation (calculated from the weight of oxide taken for the determination³) immediately rose to $+80^\circ$. The acetone solution was diluted with a relatively large amount of ether and washed six times with water. The combined water washings were titrated with 0.1 N alkali to the phenolphthalein end-point and 13.0 ml. were consumed. The reaction of the oxide with HBr used, therefore, 2.3 ml. of N HBr or 92 per cent of the theoretical amount. This is in agreement with other experiments in which the acetone solution was directly titrated with standard base. The ether was removed at room temperature, and the residue dried in an evacuated desiccator over P_2O_5 . Upon crystallization from a mixture of ether and

³ The marked dextrorotatory shift when the epoxide reacts with HBr in either acetone or acetic acid solution is probably due to the simultaneous formation of small amounts of 3(α),12-dihydroxy- $\Delta^{9,11}$ -cholenic acid or its derivatives, including the 12-acetoxy derivative, since the observed rotation of the bromohydrin is too small to account for the increase. This explanation is consistent both with the isolation of this compound from the reaction in acetic acid in the presence of H_2SO_4 and with the fact that the amount of HBr which reacts with the epoxide in either acetone or acetic acid is slightly less than 95 per cent of the theoretical amount. The fact that this side reaction takes place to a lesser extent in acetone than in acetic acid, measured by change in rotation, is to be ascribed to the greater acid strength of HBr in acetic acid. In agreement with this explanation, increasing the concentration of H_2SO_4 in acetic acid should markedly accelerate this reaction, a fact which is in accord with the observed changes in rotation.

60–70° petroleum ether the compound formed clusters of prisms, m.p. 137.5–139°; $[\alpha]_D^{22} = +54.2^\circ$ (absolute ethanol).

$C_{27}H_{43}O_5Br$. Calculated, C 61.47, H 8.26; found (A. M.), C 61.60, H 8.36

When the bromohydrin, dissolved in acetone, was titrated with aqueous alkali, the theoretical amount of base was consumed and the epoxide reformed. The product was identical with the starting material and gave no depression of melting point on admixture.

3(α), 12(α)-Dihydroxy-11(β)-bromocholan-ic Acid (II, R_1 and $R_2 = H$)—515.6 mg. of 3(α)-hydroxy-11, 12-epoxycholan-ic acid were dissolved in 49 ml. of acetone, 1.0 ml. of 48 per cent HBr added, and the mixture allowed to stand 20 minutes at room temperature. The solution was poured into ether and extracted thoroughly with water. After drying over Na_2SO_4 the ether was removed at room temperature under diminished pressure. The product crystallized from a mixture of ether and 90–100° petroleum ether. After four recrystallizations from ethyl acetate-petroleum ether, the bromohydrin formed clusters of prisms which melted sharply at 142.5°; $[\alpha]_D^{21} = +54.2^\circ$ (absolute ethanol).

$C_{24}H_{39}O_4Br$. Calculated, C 61.14, H 8.34, Br 16.95; found,⁴ C 61.04, H 8.30, Br 16.74

Methyl 3(α)-Acetoxy-11(β)-bromo-12-ketocholanate (III, $R_1 = CH_3-C(=O)-$; $R_2 = CH_3$)—210 mg. of the bromohydrin (methyl 3(α)-acetoxy-11(β)-bromo-12(α)-hydroxycholanate) were dissolved in 10 ml. of stable glacial acetic acid and 3.0 ml. of 0.56 N CrO_3 in acetic acid added. The reaction mixture stood at room temperature for 4 hours and the excess CrO_3 was reduced by the addition of methanol. The mixture was poured into ether and extracted thoroughly with water, sodium carbonate, dilute hydrochloric acid, and finally with water. Upon evaporation of the ether solution under diminished pressure at a temperature not exceeding 35°, the product crystallized. It was recrystallized from an ethyl acetate-petroleum ether mixture as well formed needles, m.p. 162–164°; $[\alpha]_D^{20} = +41.4^\circ$ (absolute ethanol).

$C_{27}H_{41}O_6Br$. Calculated. C 61.71, H 7.86, Br 15.21
Found (T. S. M.). " 61.96, " 7.82, " 14.80

Methyl 3(α)-Acetoxy-12-ketocholanate (IV, $R_1 = CH_3-C(=O)-$; $R_2 = CH_3$)—71 mg. of methyl 3(α)-acetoxy-11(β)-bromo-12-ketocholanate were dissolved in 2.5 ml. of glacial acetic acid and boiled for 1 hour under a reflux

⁴ Microanalysis by Dr. Joseph Alicino, The Squibb Institute for Medical Research.

with 50 mg. of zinc dust. After filtration the solution was diluted with ether and extracted thoroughly with sodium carbonate solution and with water. The ether was removed and the product crystallized from dilute ethanol. After one recrystallization it melted at 145–147°. Upon admixture with an authentic specimen of methyl 3(α)-acetoxy-12-ketocholanate (m.p. 150–151°) there was no depression of the melting point.

Reaction of 11,12 Epoxide with Concentrated H_2SO_4 in Acetone—Within a short time after the addition of H_2SO_4 to an acetone solution of the 11,12 epoxide there is an increase in specific rotation and parallel with this a decrease in titratable acidity (Fig. 1). The rotation falls progressively with time and concomitantly the titratable acidity increases. The prod-

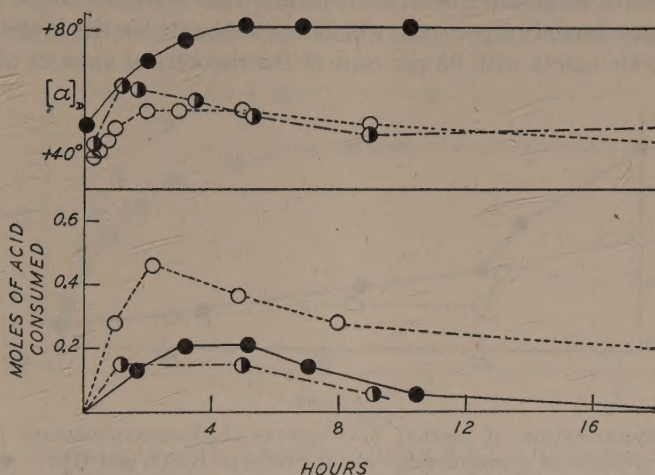


FIG. 1. Mutarotation of 11,12 epoxide in 0.07 N H_2SO_4 in acetone. ●, methyl 3(α)-acetoxy-11,12-epoxycholanate; ○, methyl 3(α)-hydroxy-11,12-epoxycholanate; ◐, 3(α)-hydroxy-11,12-epoxycholanic acid.

ucts obtained are unsaturated, as shown by titration with perbenzoic acid, and there was likewise a slight reaction with lead tetraacetate. No crystalline products were obtained.

Reaction of 11,12 Epoxide with Hydrobromic Acid in Acetic Acid—An acetic acid solution of hydrobromic acid was prepared by dissolving a carefully weighed amount of 48 per cent aqueous hydrobromic acid in glacial acetic acid. The stock solution was then diluted with glacial acetic acid to the desired molarity of HBr. The epoxide was dissolved in glacial acetic acid, slightly more than the calculated amount of the hydrobromic acid introduced, and the solution made to volume. Polariscopes readings were then taken at suitable intervals thereafter until the rotation reached a constant value (Fig. 2).

Estimations of the amount of hydrobromic acid present after reaction with the epoxide were made in the following manner. A series of dilutions of hydrobromic acid was prepared equivalent to from 1.0 to 14.0 ml. of 0.01 M HBr in 10 ml. of acetic acid. *o*-Nitroaniline was used as the indicator and a standard reference curve of the relation between color and hydrogen ion concentration constructed from the galvanometer readings of an Evelyn photoelectric colorimeter. It was necessary to have a constant concentration of indicator per ml. of solution and, for the most accurate results, to use the range between 1.0 and 7.0 ml. of 0.01 M HBr per 10 ml. of acetic acid. The accuracy of the procedure was checked by titration of the hydrobromic acid with a standard solution of anhydrous sodium acetate in glacial acetic acid; estimations could be made with an error of approximately 3 per cent, which was adequate for the investigation.

The epoxide reacts with 95 per cent of the theoretical amount of hydro-

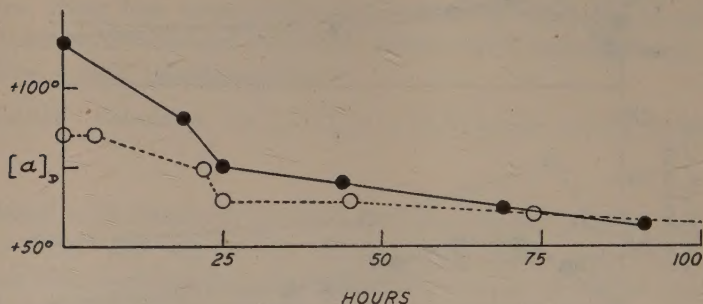


FIG. 2. Mutarotation of methyl 3(α)-acetoxy-11,12-epoxychohanate in acetic acid in the presence of a considerable molar excess of H_2SO_4 and HBr. ●, H_2SO_4 ; ○, HBr.

bromic acid, the reaction being identical with that in acetone. We did not attempt the isolation of the bromohydrin from this reaction, but a satisfactory proof for the initial formation of this compound was obtained by the isolation of methyl 3(α)-acetoxy-11(β)-bromo-12-ketocholanate. An excess of the hydrobromic acid was added to an acetic acid solution of methyl 3(α)-acetoxy-11,12-epoxychohanate and after a few minutes an excess of CrO_3 was introduced. The reaction mixture stood at room temperature for 4 hours. When the product was isolated as before, it proved to be methyl 3(α)-acetoxy-11(β)-bromo-12-ketocholanate, identical in all respects with the compound obtained by oxidation of the bromohydrin.

When methyl 3(α)-acetoxy-11,12-epoxychohanate was allowed to stand in acetic hydrobromic acid solution until the rotation had fallen to $+60^\circ$ (30 hours), it proved impossible to isolate a crystalline product.

Reaction of 11,12 Epoxide with Sulfuric Acid in Glacial Acetic Acid—In

the presence of excess sulfuric acid, the rotation rises immediately and within a short interval begins to fall again. This abrupt change is not accompanied by a change in acidity measured as before. Similar changes in rotation are observed with less than equimolar amounts of sulfuric acid. With less than equivalent amounts of sulfuric acid the reaction proceeds for about 2 hours, after which the specific rotation remains constant (Fig. 3). The products isolated from this experiment are nevertheless a mixture. Caution must be observed in working up the products, for, if the material is heated upon the steam bath, the oily residue becomes yellow and the specific rotation falls to a much lower value. This change can be prevented by removing the solvents under diminished pressure at room temperature.

593.3 mg. of methyl 3(α)-acetoxy-11,12-epoxycholanate (1.33 mm) in 10 ml. of glacial acetic acid with 0.0957 mm of sulfuric acid stood at room

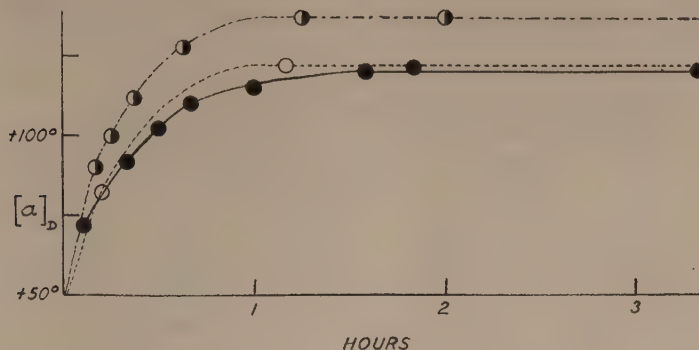


FIG. 3. Mutarotation of 11,12 epoxide in 0.01 M H_2SO_4 in acetic acid. ●, methyl 3(α)-acetoxy-11,12-epoxycholanate with 0.28 mole of H_2SO_4 per mole of epoxide; ○, methyl 3(α)-hydroxy-11,12-epoxycholanate with 0.29 mole of H_2SO_4 per mole of epoxide; ◐, 3(α)-hydroxy-11,12-epoxycholanic acid with 0.23 mole of H_2SO_4 per mole of epoxide.

temperature for $3\frac{3}{4}$ hours. The rotation at this time was $[\alpha]_D = +120^\circ$. The solution was poured into ether and washed twenty times with H_2O . The ether solution was dried over Na_2SO_4 , the ether distilled off below 28° (bath temperature), and the oily residue dried in the presence of P_2O_5 at room temperature. 605 mg. of colorless oil, $[\alpha]_D = +119^\circ$, were obtained. This material had a saponification equivalent of 190 after 2 hours boiling with base. It reacted with perbenzoic acid at room temperature, 48.4 mg. consuming 0.71 mg. of active oxygen in 24 hours.

These results indicated that the reaction resulted in part in an acetolysis of the epoxide catalyzed by hydrogen ion. Since by analogy to the reaction of the epoxide with HBr the acetoxy grouping should have been introduced at C-11, the mixture was carefully investigated for the presence of this compound. 5.4 gm. of methyl 3(α)-acetoxy-11,12-epoxycholanate were

dissolved in 200 ml. of glacial acetic acid, 4.0 ml. of 0.958 M H_2SO_4 in glacial acetic acid added, and the mixture allowed to stand at room temperature for $3\frac{1}{2}$ hours. At this time the specific rotation (calculated from the epoxide) was $+114^\circ$. The solution was poured into ether, washed with twenty successive portions of water, dried over Na_2SO_4 , and the ether removed under diminished pressure at 30° . The residual oil was thoroughly dried in a desiccator, dissolved in 70 ml. of a mixture of benzene-petroleum ether, 2:3, and poured over a column of 50 gm. of activated Al_2O_3 . The fractions collected are given in Table I.

$3(\alpha),12\text{-Dihydroxy-}\Delta^{9,11}\text{-cholenic Acid}$ ($V, R_1 = H; R_2 = H$)—Fraction 1 was rechromatographed over 50 gm. of Al_2O_3 with petroleum ether alone as the solvent. 1000 ml. of this solvent eluted in the initial fraction 500 mg. of an oily substance; $[\alpha]_D = +180^\circ$. Several other fractions were eluted, but, since the rotation was slightly lower, these were considered mixtures and were not further investigated. Two duplicate portions each con-

TABLE I
Chromatographic Separation of Acetolysis Products

Fraction No.	Solvent	Ratio	Volume	Weight of eluate	$[\alpha]_D$
			ml.	gm.	degrees
1	Benzene-petroleum ether	2:5	200	2.9	+158
2	“ “	3:1	2000	1.4	+60
3	Ether		1300	0.6	+55
4	Methanol		150	0.2	+45

taining 200.4 mg. of the oil were saponified with 0.25 N NaOH in ethanol for 2 hours under a reflux. The saponification equivalent was 208. The neutral solution was acidified under ether, and the acid extracted with ether, washed, and evaporated to dryness. Upon solution in acetone-benzene mixture crystals deposited on standing, m.p. $168\text{--}180^\circ$; $[\alpha]_D^{22} = +99.3^\circ$. Recrystallized from ethanol the compound melted at $166\text{--}170^\circ$. Recrystallized from ethyl acetate-petroleum ether it sintered at 149° and melted at $178\text{--}184^\circ$ with some preliminary softening. 2.7 mg. dissolved in 2.0 ml. of acetic acid containing a very small amount of H_2SO_4 gave a specific rotation of $+237^\circ$ when read 8 minutes after mixing.

$\text{C}_{24}\text{H}_{38}\text{O}_4$. Calculated, C 73.81, H 9.81; found (A. M.), C 74.78, H 10.43

A sample of the compound was sent to Dr. E. C. Kendall for comparison with his compound, $3(\alpha),12\text{-dihydroxy-}\Delta^{9,11}\text{-cholenic acid}$. Dr. Kendall reported that there was no depression on admixture with an authentic specimen of $3(\alpha),12\text{-dihydroxy-}\Delta^{9,11}\text{-cholenic acid}$, and, since he and his

coworkers have reported a similar marked increase in rotation when their compound was treated with mineral acid in acetic acid solution, there can be little doubt that the two substances are identical.⁵

3(α),12(α)-Dihydroxy-11(β)-acetoxycholanic Acid (VII)—Fractions 2, 3, and 4 from the initial chromatogram were combined and chromatographed over 50 gm. of Al_2O_3 . A small amount of high rotatory component was removed by elution with benzene and then 1.29 gm., $[\alpha]_D = +45^\circ$, were removed with 400 ml. of benzene-ether, 3:2. This material failed to crystallize under a variety of conditions. Two duplicate portions each containing 190 mg. were hydrolyzed by boiling with 7.0 ml. of 0.25 N NaOH in 50 per cent ethanol for 2 hours. The saponification equivalent was 279. The solution of the sodium salt was added to dilute H_2SO_4 dropwise with stirring at 50° . After cooling to room temperature, the crystalline product was filtered off and recrystallized from dilute ethanol. Needles melting at $148\text{--}160^\circ$ were obtained. After three recrystallizations from dilute ethanol the melting behavior was unchanged. After recrystallization from ethyl acetate-petroleum ether the product formed clusters of fine needles melting at $202\text{--}230^\circ$. After four further recrystallizations from this mixture the melting point remained constant at $232\text{--}233^\circ$; $[\alpha]_D^{20} = +52^\circ$ (95 per cent ethanol).

$\text{C}_{26}\text{H}_{42}\text{O}_6$. Calculated, C 69.30, H 9.40; found (A. M.), C 69.15, H 9.60

34.5 mg. dissolved in 1.6 ml. of 0.624 N KOH in 30 per cent ethanol and heated under a reflux for 3 hours consumed 0.152 ml. of N base, or a saponification equivalent of 227. Calculated for $\text{C}_{26}\text{H}_{42}\text{O}_6$ (2 equivalents), 226.

Methyl 3(α)-Acetoxy-11,12-diketocholanoate (VIII, $R_1 = \text{CH}_3\text{—}\overset{\text{O}}{\parallel}\text{C—}$; $R_2 = \text{CH}_3$)—In subsequent experiments with larger quantities of epoxide for the acetolysis a third component of the reaction mixture was separated from the lower dextrorotatory fraction. This product was more strongly adsorbed on Al_2O_3 than methyl 3(α),11(β)-diacetoxo-12(α)-hydroxycholanoate. Elution with ethyl acetate yielded an oil; $[\alpha]_D = +45^\circ$. 1.5 gm. of this product, dissolved in 20 ml. of stable glacial acetic acid, were oxidized with 30 ml. of 0.78 N CrO_3 in acetic acid at room temperature for 30 minutes. Excess CrO_3 was reduced with methanol, and the solution diluted with ether and extracted with water. The ether solution was washed with NaHCO_3 solution and with water. After drying over Na_2SO_4 , the ether

⁵ Dr. Kendall likewise informed us that 3(α),12-dihydroxy- $\Delta^9,11$ -cholenic acid forms relatively stable choleic acids with benzene, which may account for our high carbon figure. We are grateful to Dr. Kendall for his assistance.

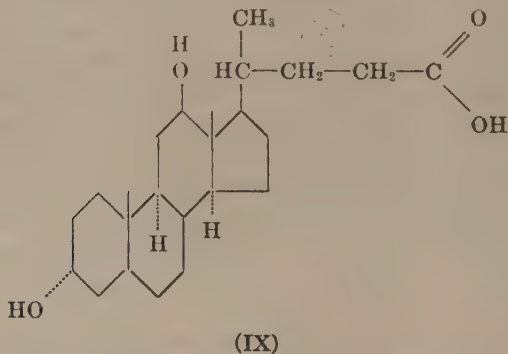
was removed and 910 mg. of neutral material obtained. This was purified by chromatographing over 15 gm. of Al_2O_3 . Elution with benzene-petroleum ether, 3:1, yielded an oily fraction which deposited 180 mg. of crystals melting at $181-186^\circ$. Further elution of the column yielded only oily products. The compound was recrystallized from ethyl acetate-petroleum ether and after four recrystallizations had a constant melting point of $197-198^\circ$; $[\alpha]_D^{21} = +129^\circ$ (ethyl acetate).

$\text{C}_{27}\text{H}_{46}\text{O}_6$. Calculated, C 70.40, H 8.75; found,⁴ C 70.51, 70.23, H 8.93, 8.81

DISCUSSION

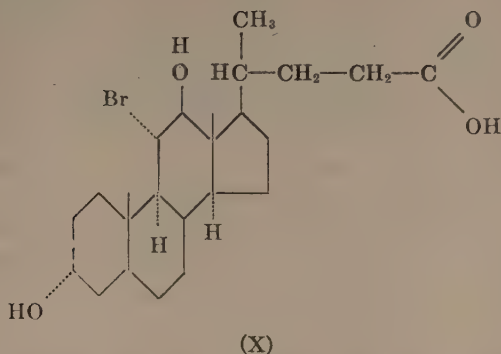
The 11,12 epoxide of lithocholenic acid obtained by means of perbenzoic acid reacts with hydrobromic acid to form a bromohydrin the structure of which was 3(α),12-dihydroxy-11-bromocholanic acid. Since the opening of an oxide ring is with few exceptions accompanied by inversion of one of the substituents, the probability is strong that in the bromohydrin the halogen atom at C-11 and the hydroxyl group at C-12 are *trans* with respect to one another. It has been demonstrated by Kendall (1) that this same epoxide can be converted to desoxycholic acid by catalytic reduction in the presence of hydrochloric acid. The results of the investigation reported here demonstrate the intermediate formation of the 11-halogen-12-hydroxy derivative in the reduction so that the hydroxyl group at C-12 of this halohydrin must have the same configuration as the C-12 hydroxyl group of desoxycholic acid.

On the basis of Giacomello's (5) x-ray measurements of desoxycholic acid Koechlin and Reichstein (4) have provisionally designated the C-12 hydroxyl group as β ; that is, *cis* with respect to the methyl group at C-10 (formula IX).



If this formulation is correct, then the bromohydrin described in this

paper should be designated 3(α),12(β)-dihydroxy-11(α)-bromocholanic acid with the structure X.



On the basis of work reported in Paper IV of this series (6), we had concluded that the C-12 hydroxyl group of desoxycholic acid has the α configuration and for this reason we had initially not assigned configuration to the halogen derivatives at C-11 and the hydroxyl group at C-12 in the present paper. After this manuscript had been prepared, however, we have had access to more recent publications of Reichstein and his collaborators which have necessitated a discussion of the configuration of these compounds.

Seebeck and Reichstein (7) have prepared two crystalline epimers from the bromination of 3(α)-acetoxy-12-ketocholanic acid. These were converted to the methyl esters which were described as follows:

	M.p. °C.	$[\alpha]_D^{20}$ (CHCl ₃) degrees
Methyl 3(α)-acetoxy-11(β)-bromo-12-ketocholamate	160-161	+37.5
" 3(α)-acetoxy-11(α)-bromo-12-ketocholamate	159-161	+47.3

For other purposes we had independently prepared these same two compounds by a different procedure and therefore both substances were available for comparison. While the two epimers differ only slightly in melting point and rotation, it seems unquestionable that the methyl 3(α)-acetoxy-11(β)-bromo-12-ketocholamate (III) which we have described in the present communication with a melting point of 162-164° and $[\alpha]_D^{20} = +41.4^\circ$ in absolute ethanol is identical with the 11(β)-bromo ester reported by Seebeck and Reichstein.⁶

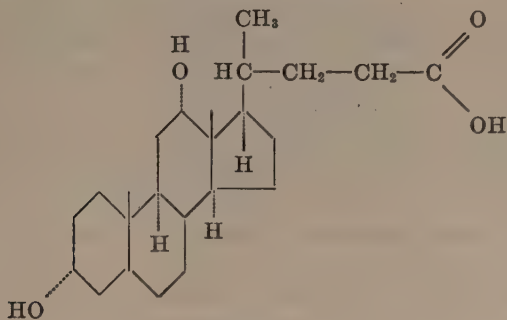
⁶ We have since verified this conclusion by dehydrobromination of the compound with pyridine. In agreement with the findings of Seebeck and Reichstein the compound is converted in excellent yield to methyl 3(α)-acetoxy-12-keto- Δ^9 ,¹¹-cholamate (m.p. 148-149°; $\epsilon_{2400} = 10,600$) after 3 hours heating. The 11(α) epimer is recovered unchanged under these conditions.

Seebeck and Reichstein assigned the β configuration to the halogen at C-11 of the compound under discussion after comparison of the ease of dehydrobromination of the two bromo ketones epimeric at C-11. The 11(β)-bromo compound readily lost HBr to form methyl 3(α)-acetoxy-12-keto- $\Delta^{9,11}$ -cholenate when it was warmed with pyridine, whereas the 11(α) compound was recovered unchanged from the same treatment. It is to be expected that a *trans* configuration of the hydrogen atom at C-9 and the halogen at C-11 would favor the removal of these substituents as HBr. Since it is most probable (8) that the hydrogen at C-9 is *trans* to the angle methyl group at C-10, the epimer which was readily converted to the α,β -unsaturated keto acid was assigned the β configuration at C-11. In Paper IV of this series (6) we shall offer further evidence for the validity of the configuration of the halogen at C-11. We therefore believe that certainty can be attached to the configuration which Seebeck and Reichstein have given the two C-11 epimers of methyl 3(α)-acetoxy-11-bromo-12-ketocholocate. For the further discussion then we shall assume that the configuration of the halogen at C-11 is established.

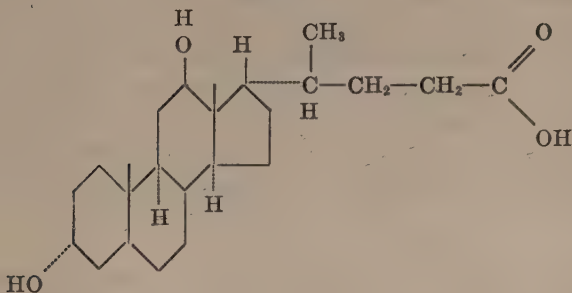
Therefore, if the structure of the bromohydrin obtained by treatment of the epoxide of Δ^{11} -lithocholenic acid is 3(α),12(β)-dihydroxy-11(β)-bromocholanic acid, either of two conclusions must be drawn. These are (a) that the opening of the epoxide has occurred without inversion or (b) that the configuration of the hydroxyl at C-12 has been incorrectly assigned by Koechlin and Reichstein (4). The first conclusion, while not impossible, is unlikely. It is therefore necessary to review the evidence which bears upon the second conclusion. Koechlin and Reichstein (4) have presented valuable information in this regard. Comparison of the velocity of saponification of the two epimeric methyl 3-keto-12-acetoxycholates established the fact that the ester epimeric at C-12 was much more readily hydrolyzed than the analogous compound in which the C-12 hydroxyl had the configuration of that of desoxycholic acid. The length of the side chain exerts considerable influence on the velocity of saponification of the ester at C-12, as shown by comparison of the cholanic acid derivatives with the corresponding etio acid (9) which is considerably more readily saponified. These authors (4) state then, "Dieser starke Einfluss der Seitenkette wäre am besten verständlich, wenn sich die 12-ständige Hydroxylgruppe und die Methingruppe Nr. 17 in *cis*-Stellung zueinander befinden (also OH und Methyl Nr. 18 *trans*-ständig)..."

These authors likewise attempted to prepare a δ -lactone from bisnor-desoxycholic acid by heating it in tetralin and by vacuum distillation at 300°. They were unsuccessful in these attempts, and, although they were unwilling to assign a definite configuration to the C-12 hydroxyl group and the side chain on the basis of a negative result, they nevertheless

indicate that their experimental findings are in agreement with the formulation of desoxycholic acid as either XI or XII in which the hydroxyl group at C-12 and the side chain at C-17 are in *trans* position to each other. Despite these results, the Swiss investigators provisionally accepted the formulation of Giacomello and have adopted the β configuration for the C-12 hydroxyl group of desoxycholic acid (XII).



(XI)



(XII)

Our experimental results are in better agreement with the conclusion that the C-12 hydroxyl group of desoxycholic acid has the α configuration and there is no contradictory evidence from Reichstein's work. The 11,12 epoxide with HBr yields a 3(α),12-dihydroxy-11(β)-bromocholanic acid which can be catalytically reduced to desoxycholic acid (1). Since the opening of the oxide should yield substituents at C-11 and C-12 in *trans* configuration to each other and since the halogen at C-11 has been shown to have the 11(β) configuration, it follows that the hydroxyl at C-12 of desoxycholic acid has α configuration. Therefore the correct structure of desoxycholic acid, 3(α),12(α)-dihydroxycholanolic acid, is shown in XI.

Accordingly, then, the bromohydrin obtained by opening the epoxide ring is 3(α),12(α)-dihydroxy-11(β)-bromocholanic acid (II), and, since the opening of the epoxide ring should proceed in the same fashion with

acetic acid as with hydrobromic acid, the amorphous product of acetolysis should be methyl 3(α),11(β)-diacetoxy-12(α)-hydroxycholanate (VI). Examination of molecular models reveals that this is the more hindered configuration at C-11, which is in accord with our finding that 2 hours hydrolysis with boiling 0.25 N NaOH yielded the crystalline 3(α),12(α)-dihydroxy-11(β)-acetoxycholanate (VII).

The formation of both methyl 3(α)-acetoxy-12-hydroxy- $\Delta^{9,11}$ -cholenate (V) and the glycol, methyl 3(α)-acetoxy-11,12-dihydroxycholanate, as well as the acetolysis product, methyl 3(α),11(β)-diacetoxy-12(α)-hydroxycholanate (VI), in the reaction of the epoxide with acetic acid in the presence of H₂SO₄ indicates the complexity of the reaction. These substances, however, represent the principal products. The formation of methyl 3(α)-acetoxy-12-hydroxy- $\Delta^{9,11}$ -cholenate is quite readily explained on the basis of the configuration we have assigned to the compounds formed by the fission of the epoxide ring. If the elements either of water or of sulfuric acid are added to the epoxide, the substituent at C-11 is *trans* to the hydrogen at C-9 and is more labile in this configuration. It is to be expected that the elements of water or sulfuric acid would be readily lost under the influence of the sulfuric acid present in the mixture. With higher acid concentration this reaction should take place more rapidly and completely. A strongly dextrorotatory substance, very probably methyl 3(α)-acetoxy-12-hydroxy- $\Delta^{9,11}$ -cholenate, was the principal reaction product in the presence of higher concentrations of strong acid. This finding is further evidence for the configuration we have assigned to the substituents at C-11.

We wish to express our appreciation of the technical assistance given us by Miss Joanna Xenos.

SUMMARY

1. The 11,12 epoxide formed from Δ^{11} -lithocholenic acid has 11(α),12(α) configuration.
2. The epoxide reacts with hydrobromic acid to form a bromohydrin in which the halogen is at C-11.
3. The configuration assigned this compound is 3(α),12(α)-dihydroxy-11(β)-bromocholanate acid.
4. The configuration of the C-12 hydroxyl group of desoxycholic acid is discussed and the conclusion drawn that this has the α configuration or *trans* to the angle methyl groups at C-10 and C-13.
5. When the 11,12 epoxide of Δ^{11} -lithocholenic acid reacts with acetic acid in the presence of sulfuric acid, at least three compounds are formed.

These have been identified as 3(α),12-dihydroxy- $\Delta^{9,11}$ -cholenic acid, 3(α),11,12-trihydroxycholanolic acid, and 3(α),12(α)-dihydroxy-11(β)-acetoxycholanolic acid.

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PARTIAL SYNTHESIS OF COMPOUNDS RELATED TO ADRENAL CORTICAL HORMONES

PREPARATION OF 3(α),11(α)-DIHYDROXYCHOLANIC ACID*

By WILLIAM P. LONG

In Paper II we (1) have shown that methyl 3(α)-acetoxy-11,12-epoxycholesterol undergoes fission of the epoxide ring with the introduction of the anionic constituent at C-11. The products of such reactions offered, we felt, a possible route to the preparation of 3(α),11-dihydroxycholesterol acid.

Marker and Lawson (2) prepared 3(α),11-dihydroxy-12-ketocholanic acid by bromination and subsequent alkaline hydrolysis of 3(α)-acetoxy-12-ketocholanic acid. They could not reduce the product by the Clemmensen method but were able to obtain a crude semicarbazone. They state that Wolff-Kishner reduction was also unsuccessful, although no details of procedure or products were given. Longwell and Wintersteiner (3) reinvestigated the problem and were unable to secure the semicarbazone described by Marker and Lawson. These authors could not obtain either a hydrazone or an oxime and upon treatment of the 3(α),11-dihydroxy-12-ketocholanic acid with sodium ethylate and hydrazine hydrate at 200° both oxygen atoms in Ring C were eliminated. Marker, Shabica, Jones, Crooks, and Wittbecker (4) repeated the reduction of 3(α),11-dihydroxy-12-ketocholanic acid, using the method employed by Longwell and Wintersteiner, and obtained a product characterized as 3(α),11,12-trihydroxycholesterol acid. Although it appeared from these results that the reduction of a 12-keto bile acid with a hydroxyl group at C-11 would not yield the desired product, there were at least two reasons for investigating this problem. It was possible (1) that the structure of the compound prepared by Marker and Lawson was not that assigned by these authors, or (2) that, if the structure were correct, the epimeric compound with the C-11 hydroxyl group in the opposed configuration would react with ketonic reagents and permit reduction of the carbonyl group. The experiments recorded here were undertaken to explore these possibilities.

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

Since the preparation of methyl 3(α),11(β)-diacetoxy-12(α)-hydroxy-cholanate by acetolysis of the 11,12 epoxide gave poor yields, a series of experiments was performed in an attempt to improve the conditions. Methyl 3(α)-acetoxy-11,12-epoxycholanate reacts with acetic acid at 100° in the absence of mineral acid, as measured by the change in rotation, but, since much unchanged epoxide could be recovered after 1 to 2 hours heating, it appeared that these conditions favored the formation of methyl 3(α)-acetoxy-12-hydroxy- $\Delta^{9,11}$ -cholenate (or the 12-acetate of this compound) and this method was abandoned in favor of the acid-catalyzed reaction described in Paper II (1).

Trial experiments in which rotation alone was measured gave the following results: When higher concentrations (0.02 M) of anhydrous perchloric acid were added to an acetic acid solution of the epoxide, the rotation increased to +170° immediately; with smaller concentrations (0.002 M) the same rotation was observed after 1 hour at room temperature. These results demonstrated that the conversion of the epoxide to the $\Delta^{9,11}$ acid was more rapid at high hydrogen ion concentration than the acetolysis. A series of experiments was carried out with small amounts of sulfuric acid (1.2×10^{-4} M), the solution of the epoxide being heated to boiling for from 10 to 20 minutes. The procedure gave relatively large amounts (50 to 60 per cent) of methyl 3(α)-acetoxy-12-hydroxy- $\Delta^{9,11}$ -cholenate and from 15 to 20 per cent of methyl 3(α)-acetoxy-11,12-dihydroxycholanate, while the yield of the desired methyl 3(α),11(β)-diacetoxy-12(α)-hydroxy-cholanate was small and variable. When the reaction was studied at 0° with higher acid concentration (1.25×10^{-2} to 4.6×10^{-2} M), the formation of methyl 3(α)-acetoxy-12-hydroxy- $\Delta^{9,11}$ -cholenate was found to be less than when the reaction was conducted at higher temperatures. The yield of the acetolysis product was not markedly higher when calculated from the amount of epoxide used for the experiment. Since, however, some 40 to 50 per cent of unchanged oxide could be recovered after 18 hours, this appeared to be a more economical procedure and a minor modification of these conditions was used for the acetolysis. A typical procedure is described in the experimental section. These experiments were complicated by our failure to obtain methyl 3(α),11(β)-diacetoxy-12(α)-hydroxycholanate in a crystalline state, and, for this reason, estimates of the yield of this compound were based on the amount of crystalline methyl 3(α),11(β)-diacetoxy-12-ketocholanate obtained after chromic acid oxidation.

An alternative method for the preparation of an 11-acetoxy derivative in which methyl 3(α)-acetoxy-11(β)-bromo-12-ketocholanate was treated with potassium acetate in methanol solution yielded only unchanged

starting material and small amounts of amorphous product. The action of silver acetate on methyl 3(α)-acetoxy-11(β)-bromo-12(α)-hydroxycholanate was investigated. A portion of the material was converted to the 11,12 epoxide, while the remainder underwent loss of HBr with the formation of methyl 3(α)-acetoxy-12-hydroxy- $\Delta^9,^{11}$ -cholenate.

EXPERIMENTAL¹

Reaction of Methyl 3(α)-Acetoxy-11(β)-bromo-12(α)-hydroxycholanate with Silver Acetate—3.0 gm. of methyl 3(α)-acetoxy-11,12-epoxycholanate were dissolved in 50 ml. of acetone and 8.0 ml. of 1.5 N HBr added. After 15 minutes the solution was neutralized with 11.5 ml. of 0.5 N NaOH. The bromohydrin was extracted with ether, washed with water, and, after drying over Na_2SO_4 , the ether was removed under diminished pressure at 30°. The oily residue was thoroughly dried in the desiccator, dissolved in 200 ml. of glacial acetic acid, and shaken mechanically for 15 hours with 1.9 gm. of powdered silver acetate. The solution was decanted into ether and washed with water, dilute HNO_3 , 5 per cent NaHCO_3 solution, and finally with water. After removal of the ether and crystallization from dilute methanol 1.84 gm. melting at 142–143° were obtained. No depression was found on admixture with methyl 3(α)-acetoxy-11,12-epoxycholanate. When dissolved in acetic acid in the presence of H_2SO_4 , the compound showed the typical mutarotation of the epoxide.

The mother liquors yielded a second crop of 550 mg. of crystals, m.p. 81–85°, which on recrystallization from dilute acetone melted unsharply at 83–106°; $[\alpha]_D^{24} = +105^\circ$ (acetic acid). When a trace of concentrated H_2SO_4 was added, the rotation promptly shifted to $[\alpha]_D^{24} = +210^\circ$. 46.2 mg. in 1.0 ml. of absolute ethanol and 1.0 ml. of 1.5 N KOH were boiled under a reflux for 4 hours and a saponification equivalent of 228 was obtained; calculated for $\text{C}_{27}\text{H}_{42}\text{O}_5$ (2 equivalents), 224. Both the optical behavior and the saponification are consistent with methyl 3(α)-acetoxy-12-hydroxy- $\Delta^9,^{11}$ -cholenate.

Methyl 3(α)-Acetoxy-12-keto- $\Delta^9,^{11}$ -cholenate—50 mg. of methyl 3(α)-acetoxy-12-hydroxy- $\Delta^9,^{11}$ -cholenate were dissolved in 1.0 ml. of pure glacial acetic acid, 0.5 ml. of 1.32 N CrO_3 in acetic acid was added, and the mixture stood at room temperature 20 minutes. Methanol was added and the solution diluted with water. The ether was washed with water, 5 per cent NaHCO_3 solution, and again with water. Removal of the ether

¹ All melting points are corrected. The microanalyses were performed by Dr. Amel Menotti (A. M.), American Medical Association, Chicago, and by Dr. Joseph Alicino (J. A.) of The Squibb Institute for Medical Research, New Brunswick, New Jersey. We wish to express our appreciation for this service.

yielded 44 mg. of crystals, m.p. 143–145°, After four recrystallizations from methanol it formed rectangular prisms, m.p. 149–150°.

$C_{27}H_{40}O_6$. Calculated, C 72.77, H 9.05; found (J. A.), C 72.31, H 8.64

The product had the characteristic absorption spectrum of an α,β -unsaturated ketone; $\epsilon_{2390} = 11,700$.

Methyl 3(α),11(β)-Diacetoxy-12-ketocholanate—190 mg. of methyl 3(α),11(β)-diacetoxy-12-hydroxycholanate (amorphous, $[\alpha]_D = +45^\circ$), obtained by the method used in Paper II (1), were dissolved in 3.0 ml. of pure glacial acetic acid, 7.0 ml. of 0.53 N CrO_3 in acetic acid added, and the reaction mixture cooled to between 5–10° for 2 hours. The excess CrO_3 was reduced with methanol, and the solution poured into ether and separated into acidic and neutral fractions in the usual manner. The neutral fraction yielded 68 mg. of crystals from ether-petroleum ether, which after one recrystallization from petroleum ether melted at 109–111°; $[\alpha]_D^{20} = +124^\circ$ (absolute ethanol).

$C_{29}H_{44}O_7$. Calculated. C 69.02, H 8.83, saponification equivalent 170
Found (A. M.). " 69.35, " 9.18, " " 169

Oxime of Methyl 3(α),11(β)-Diacetoxy-12-ketocholanate—24 mg. of methyl 3(α),11(β)-diacetoxy-12-ketocholanate were heated under a reflux with 35 mg. of hydroxylamine hydrochloride and 68 mg. of sodium acetate trihydrate in 5.0 ml. of ethanol for 5 hours. Upon dilution with water very fine needles were deposited which were twice recrystallized from ethyl acetate-petroleum ether, m.p. 136–138°.

$C_{29}H_{45}O_7N$. Calculated, N 2.71; found,² 2.51

No semicarbazone was found either by heating for 5 hours with semicarbazide acetate in pyridine-alcohol solution or by standing at room temperature for several weeks with semicarbazide acetate in pyridine-alcohol solution. Under the latter conditions a small amount of unchanged starting material was recovered but no other crystalline product could be found.

Hydrazone of 3(α)-Hydroxy-11(β)-acetoxy-12-ketocholanhydrazide—200 mg. of methyl 3(α),11(β)-diacetoxy-12-ketocholanate were heated under a reflux for 15 hours with 0.5 ml. of hydrazine hydrate and 1.0 ml. of absolute ethanol. The mixture was cooled in an ice bath; 190 mg. of crystals were obtained which were recrystallized five times from 95 per cent ethanol. The compound was difficult to purify but finally came to a constant

² Microanalysis by Dr. T. S. Ma, Department of Chemistry of the University of Chicago, Chicago.

melting point of 221–223° in a capillary tube. When the melting point was observed under a microscope, the compound melted over 8° in this range. It is noteworthy that, while the prolonged treatment with hydrazine removed the ester groups at C-3 and C-24, the acetoxy group at C-11 was resistant to the reagent. It is possible that epimerization or partial removal of the acetoxy group was responsible for the difficulty in purification.

$C_{26}H_{44}O_4N_4 \cdot H_2O$. Calculated. C 63.13, H 9.37, N 11.32
Found (A.M.). " 63.33, " 9.89, " 11.82

3(α), 11(β)-Dihydroxy-12-ketocholanic Acid—521 mg. of methyl 3(α), 11(β)-diacetoxy-12-ketocholanate were dissolved in 5.0 ml. of 95 per cent ethanol and 11.7 ml. of 0.6 N KOH in 85 per cent ethanol added. The solution was chilled to 0°. After 3 hours the flask was filled with crystals which after 24 hours at this temperature were filtered, washed with a small amount of cold ethanol, and dried. Yield 414 mg. or 90 per cent of theory. After one recrystallization from water the salt melted at 223–226°; $[\alpha]_D^{25} = +90^\circ$ (0.36 per cent solution in absolute ethanol).

The potassium salt was converted to the acid with acetic acid. This compound proved extremely difficult to crystallize. It forms clumps of soft needles from a concentrated solution in methanol. The best melting point obtained was 147–157°; because of this no analysis was performed. The methyl ester, prepared with diazomethane, proved similarly difficult to crystallize and melted at 189–193°.

Wolff-Kishner Reduction of Hydrazone of 3(α)-Hydroxy-11(β)-acetoxy-12-ketocholanhydrazide—100 mg. of the hydrazide hydrazone were heated at 200° in a sealed tube with 6 ml. of 5 per cent sodium ethylate and 3 drops of hydrazine hydrate for 8 hours. The product obtained on acidification of the reaction mixture was a white amorphous solid weighing 75 mg. As it appeared to be inhomogeneous, it was converted to the methyl ester with diazomethane. The esters were separated by chromatographing on 3 gm. of activated Al_2O_3 according to the scheme given in Table I. Each fraction represented 50 ml. of eluate.

Methyl 3(α)-Hydroxy- Δ^{11} -cholenate—The 24 mg. eluted with benzene-ether were recrystallized from petroleum ether and melted at 96–100°; $[\alpha]_D^{22} = +50^\circ$ (ethyl acetate). Upon admixture with an authentic sample of methyl 3(α)-hydroxy- Δ^{11} -cholenate (m.p. 101–106°) there was no depression of the melting point. It is probable that this product was nevertheless a mixture of methyl lithocholenate and methyl lithocholate similar to that described in Paper IV (5), since these two compounds form mixed crystals.

Methyl 3(α), 11(α)-Dihydroxycholanate—19 mg. of the crystalline material eluted with ethyl acetate were recrystallized three times from ethyl acetate-

petroleum ether. The compound formed clumps of long pointed needles melting at 132–133°; $[\alpha]_D^{22} = +19^\circ$ (ethyl acetate).

$C_{25}H_{42}O_4$. Calculated, C 73.85, H 10.41; found (A. M.), C 73.67, H 10.56

3(α),11(α)-Dihydroxycholanolic Acid—212 mg. of methyl 3(α),11(α)-dihydroxycholanate were hydrolyzed for 1 hour on the steam bath with 5 ml. of 0.5 N alcoholic NaOH. The solution was poured slowly into dilute H_2SO_4 with stirring, the precipitate filtered, and after one crystallization from ethyl acetate-petroleum ether, melted at from 120–145°. After three recrystallizations from the same solvent the product sintered to an opaque

TABLE I
Separation of Products from Wolff-Kishner Reduction

Solvent	Ratio	Weight eluted	Description
		mg.	
Benzene-petroleum ether	1:1	2.4	Oily
“ “	3:1	0.5	“
Benzene		1.0	“
Benzene-ether	9:1	0.5	“
“	4:1	14.0	Crystalline
“	3:2	10.0	“
“	3:2	0.5	Oily
“	3:2	0.5	“
“	2:3	1.0	“
“	1:4	1.3	“
Ether		1.0	“
“		2.8	“
Ethyl acetate		19.0	Crystalline

mass at 127° and slowly became clear up to 147°; $[\alpha]_D^{25} = +22^\circ$ (absolute ethanol).

$C_{24}H_{40}O_4$. Calculated, C 73.43, H 10.27; found (J. A.), C 73.98, H 10.62

Methyl 3(α),11(α)-Diacetoxycholanate—46 mg. of methyl 3(α),11(α)-dihydroxycholanate were heated in a sealed tube at 100° for 1 hour with 0.5 ml. of acetic anhydride and 0.5 ml. of pyridine. The mixture was poured into ice and dilute sulfuric acid, and allowed to stand overnight. The semicrystalline product, recrystallized from dilute methanol, melted at 105–111°. After two recrystallizations from the same solvent the melting point was 118–119°; $[\alpha]_D^{25} = +13.4^\circ$ (ethyl acetate), $[\alpha]_D^{20} = +7.0^\circ$ ($CHCl_3$).

$C_{29}H_{46}O_6$. Calculated. C 70.98, H 9.45, saponification equivalent 163
Found (A. M.). “ 71.20, “ 9.46, “ “ 167

Attempted Dehydration at C-11—Removal of the C-11 hydroxyl group was attempted by the method which Shoppee (6) found to be successful for the cortical substances. 27 mg. of methyl 3(α),11(α)-dihydroxycholanate in 0.4 ml. of glacial acetic acid and 0.1 ml. of concentrated HCl were heated under a reflux for 30 minutes. The product was esterified with diazomethane and acetylated with acetic anhydride in pyridine. Only methyl 3(α),11(α)-diacetoxycholanate (21 mg. of crystalline material, m.p. 109–112°) was obtained. This product gave no depression of melting point when mixed with an authentic sample of methyl 3(α),11(α)-diacetoxycholanate, but, when mixed with methyl 3(α)-acetoxyl- Δ^{11} -cholanate (m.p. 115–117°), the melting point was depressed to 94–97°.

Methyl 3,11-Diketocholanate—25 mg. of slightly impure methyl 3(α),11(α)-dihydroxycholanate were dissolved in 1.0 ml. of pure glacial acetic acid, 1.0 ml. of 0.6 N CrO_3 in acetic acid added, and the mixture left at room temperature for 20 hours. The acetic acid was removed under diminished pressure at 30° and the residue dissolved in water and ether. The ether solution was washed with dilute sulfuric acid, sodium bicarbonate solution, and water, and then dried over Na_2SO_4 . After removal of the ether, the resulting yellow oil was purified by chromatographing over 2 gm. of activated Al_2O_3 . 7.0 mg. were eluted with benzene-ether, 4:1, after several small oily fractions had been discarded. This product crystallized in square plates from 60–70° petroleum ether after standing. It melted at 83–85°. Subsequently, when larger amounts were prepared, recrystallization from 60–70° petroleum ether yielded hexagonal plates melting at 84–85°; $[\alpha]_D^{25} = +69^\circ$ (ethyl acetate). Lardon and Reichstein (7) report a melting point of 82–84°; $[\alpha]_D^{17} = +61.7^\circ$ (acetone).

$\text{C}_{25}\text{H}_{38}\text{O}_4$. Calculated, C 74.59, H 9.51; found (J. A.), C 74.40, H 9.30

Monosemicarbazone of Methyl 3,11-Diketocholanate—70 mg. of methyl 3,11-diketocholanate (m.p. 86°) were dissolved in 3.0 ml. of ethanol and 5 ml. of a solution of semicarbazide acetate prepared from 106 mg. of semicarbazide hydrochloride and 126 mg. of sodium acetate trihydrate were added. The mixture stood 16 hours at room temperature and the product was precipitated by the addition of water. Four recrystallizations from methanol yielded rectangular plates melting at 194–197°.

$\text{C}_{25}\text{H}_{40}\text{O}_4\text{N}_3$. Calculated, N 9.41; found (J. A.), 9.49

Improved Acetolysis of Methyl 3(α)-Acetoxy-11,12-epoxycholanate—10 gm. of methyl 3(α)-acetoxy-11,12-epoxycholanate were dissolved in 300 ml. of anhydrous acetic acid and 200 ml. of toluene. 6.0 ml. of 0.96 M H_2SO_4 in acetic acid were added, and the flask immediately chilled to 0° and maintained at this temperature for 16 hours. The solution was poured into

ether, extracted with water and 10 per cent Na_2CO_3 solution, and, after drying over Na_2SO_4 , the solvent was removed at 30° . The residue, weighing 10.3 gm., was dissolved in 50 ml. of pure glacial acetic acid. 100 ml. of 1.0 N CrO_3 in acetic acid were then added and, after 15 minutes at room temperature, the excess CrO_3 was reduced by addition of methanol. The mixture was poured into a large volume of water and ether and the ether solution washed successively with water, Na_2CO_3 solution, and water. The ether was removed at 30° and the neutral residue (weight 8.4 gm.) in 150 ml. of benzene-petroleum ether, 3:1, was poured over 100 gm. of activated alumina. The fractions given in Table II were eluted.

Fractions 5 and 6 were combined and after recrystallization yielded 1.70 gm. of methyl 3(α),11(β)-diacetoxy-12-ketocholanate, m.p. $107-110^\circ$.

TABLE II
Separation of Products from Acetolysis after CrO_3 Oxidation

Fraction No.	Solvent*	Ratio	Weight of eluate gm.	Description
1	Benzene-petroleum ether	3:1	0.399	Oily
2	Benzene		0.987	Crystalline; almost pure
3	Benzene-ether	20:1	2.037	methyl 3(α)-acetoxy-11,-
4	"	10:1	0.894	12-epoxycholanate
5	"	3:1	1.374	Crystalline
6	"	2:1	0.750	"
7	Ether		0.075	Oily
8	Methanol		1.695	"

* Each fraction represented 1 liter of eluate except Fractions 7 and 8 which were 500 ml.

DISCUSSION

From these experiments, the significant facts emerge that an 11(β)-hydroxyl group does not prevent the formation of ketonic derivatives at C-12 and that the ketone group can be successfully reduced to a methylene without loss of the hydroxyl at C-11. The failure of the 3,11-dihydroxy-12-ketocholanic acid described by Marker and Lawson to form ketonic derivatives is due to rearrangement by the alkaline hydrolysis involved in its preparation, as will be discussed in Paper VI (8) of this series.

The 3,11-dihydroxycholanolic acid isolated by Wolff-Kishner reduction of methyl 3(α),11(β)-diacetoxy-12-ketocholanate has been assigned the 11(α) configuration on the basis of the ease of acetylation and the relative resistance to dehydration of the C-11 hydroxyl group. It is interesting to note that whereas the 11(α)-hydroxyl group is sterically unhindered the ketone group at this position is unreactive as demonstrated by the forma-

tion of a monosemicarbazone from methyl 3,11-diketocho lanate. The ketonic group therefore resembles the inert ketonic group at C-11 of the cortical steroids. The cortical steroids must have the hydroxyl group at C-11 in the β configuration, since these substances have been shown to be resistant to acylation and are, moreover, readily dehydrated with mineral acids.

The mechanism of formation of 3(α),11(α)-dihydroxycho lanic acid by the Wolff-Kishner reduction will be discussed in Paper IV (5).

We wish to express our appreciation of the technical assistance of Miss Joanna Xenos.

SUMMARY

1. Methyl 3(α),11(β)-diacetox y-12-ketocho lanate was prepared from methyl 3(α)-acetox y-11,12-epox ycho lanate by acetolysis and oxidation with CrO_3 .

2. This compound readily forms an oxime and a hydrazone.

3. Wolff-Kishner reduction yielded an equal amount of a compound tentatively identified as Δ^{11} -lithocho lenic acid and 3(α),11(α)-dihydroxycho lanic acid. The latter product has the C-11 hydroxyl group in the unhindered configuration, since it forms a diacetate under mild conditions and is not readily dehydrated by HCl in acetic acid.

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